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Adipocyte nuclei isolation from iWAT

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An abbreviated version of this protocol was published in eLIFE in Oct 2019

Single cell analysis reveals immune cell–adipocyte crosstalk regulating the transcription of thermogenic adipocytes

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Detailed protocol

Adipocyte nuclei isolation from iWAT- 200-400 mg of inguinal white adipose tissues (iWAT) from mice exposed to conditions mentioned in the text were placed on sterile 6-well tissue culture plate with ice-cold 1XPBS. Fat pads were blotted on a napkin to removed excess liquid. Tissues were cutand mincedwith scissors andwere placed in 15ml conical tubes containing digestion buffer (DPBS and Collagenase D at 9.8 mg/ml; Sigma, MO)at incubated at 37 □C for 45 mins with gentle shaking at 100 rpm. 10 ml of resuspension media (DMEM/F12 with glutamax supplemented with 15% FBS and 1% pen/strep; Thermo Scientific, CA) was added to digested solution and slowly inverted 5 times. The digestion mixture was centrifuged at 200 x g for 5mins at RT. Floating adipocytes were collected using P1000 pipet with half cut P1000 tip. Adipocytes were transferred to a new 15 ml tube and kept on ice for 5 mins. Excess liquid was aspirated using 1 ml syringe and adipocytes were then washed with 1ml DPBS and the suspension was spun down at 200 g for mins at RT. Spun down liquid was aspirated using 1 ml syringe and adipocyte nuclei were isolated using Minute nuclei and cytosol isolation kit for adipose tissue using manufacture's instruction (Invent Biotechnologies, MN) with modifications. Briefly, adipocytes were slowly resuspended in 600 □l nuclei lysis buffer (N/C Buffer) and lysate was transferred to a filter cartridge with collection tube and incubated at -20°C freezer for 20 min with cap open. After incubation, the tube was centrifuged at 2000 rpm for 2 min at 4 °C. The filter cartridge was discarded without agitation and the collection tube was immediately centrifuged at 4000 rpm for 4 min at 4 °C. Supernatant was gently removed using P200 pipet without touching the side walls. Nuclei were resuspended in 30 ul of nuclei resuspension buffer (DPBS+0.1%BSA) per 200-400 mg of iWAT (i.e. one 8-10 week chow fed mouse). For SNAP-seq, 2-3 mice were combined and 60 □l of nuclei suspension was transferred to a new 2 ml tube and resuspended with 500-700 □l of nuclei resuspension buffer and filtered using 40 □m cell strainer (Flowmi Cell Strainer, Belart, NJ) twice to get clean single nuclei suspension. As shown in Figure 3A, for quality control, nuclei were first DAPI stained and then filtered or FACS sorted to get single nuclei suspension. After microfluidic partitioning in 10xGenomics platform (see below), nuclei lysis was checked by observing oil emulsion under fluorescent microscope for DAPI diffusion.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Rajbhandari, P. (2020). Adipocyte nuclei isolation from iWAT. Bio-protocol Preprint. bio-protocol.org/prep225.
2. Rajbhandari, P., Arneson, D., Hart, S. K., Ahn, I. S., Diamante, G., Santos, L. C., Zaghari, N., Feng, A., Thomas, B. J., Vergnes, L., Lee, S. D., Rajbhandari, A. K., Reue, K., Smale, S. T., Yang, X. and Tontonoz, P.(2019). Single cell analysis reveals immune cell–adipocyte crosstalk regulating the transcription of thermogenic adipocytes. eLIFE. DOI: 10.7554/eLife.49501